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RESEARCH ARTICLE

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Effects of brain-derived neurotrophic factor on neuronal structure of dopaminergic neurons in dissociated cultures of human fetal mesencephalon

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Abstract Brain-derived neurotrophic factor (BDNF) has been shown to promote the survival of cultured fetal mesencephalic dopaminergic neurons of rat and human origin. In the present study, BDNF was tested for its ability to influence neuronal structure of dopaminergic neurons in dissociated cultures of human fetal ventral mesencephalon after 7 days in vitro. Following immunocytochemical staining for tyrosine hydroxylase, all surviving dopaminergic neurons were counted. Computer-assisted three-dimensional reconstructions of uniform randomly selected neurons cultured with 50 ng/ml BDNF (n=120) or without BDNF (n=80) were made. BDNF increased the number of surviving human dopaminergic neurons by 76%. Mean soma profile area was significantly enlarged by 18% in BDNF-treated neurons as compared to controls. Analysis of parameters of neuritic size and complexity in these cultures revealed that combined neuritic length, combined neuritic volume, and neuritic field area were increased by 60%, 125% and 129%, respectively, and the mean number of segments per cell was increased by 41%. A change in neurite complexity in BDNF-treated cultures was further confirmed by the Sholl's concentric sphere analysis. These results demonstrate that BDNF promotes development and differentiation of human fetal dopaminergic neurons in vitro.

Key words Nerve growth factors · Neuronal plasticity · Substantia nigra · Tyrosine hydroxylase · Image analysis · Human

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Introduction

Brain-derived neurotrophic factor (BDNF; Barde et al. 1982; Leibrock et al. 1989) is a member of the neurotrophin family, which also includes nerve growth factor (NGF; Levi-Montalcini 1987), neurotrophin-3 (NT-3; Ernfors et al. 1990; Hohn et al. 1990; Maisonpierre et al. 1990), neurotrophin-4/5 (NT-4/5; Berkemeier et al. 1991; Hallböök et al. 1991; Ip et al. 1992) and neurotrophin-6 (NT-6; Götz et al. 1994). These structurally and functionally related trophic molecules have potent effects on the survival, differentiation and regeneration of many classes of neurons in both the peripheral and the central nervous system (CNS; for review, Thoenen 1991; Eide et al. 1993; Korsching 1993; Lindsay et al. 1993). The tropomyosin receptor-related tyrosine kinase (Trk) family, which includes TrkA, TrkB and TrkC, has been identified as consisting of high-affinity receptors for the neurotrophins (for review, Glass and Yancopoulos 1993). BDNF utilises TrkB, which also serves as functional high-affinity receptor for NT-4/5 and, to a lesser extent, for NT-3. Levels of BDNF messenger RNA (mRNA) in striatal tissue are low (Maisonpierre et al. 1990). A comparatively high expression of BDNF and TrkB mRNA in the substantia nigra (Gall et al. 1992; Merlio et al. 1992; Seroogy et al. 1994) and a responsiveness of nigral dopaminergic neurons in cultures to BDNF (Hyman et al. 1991; 1994; Knüsel et al. 1991) are consistent with a mainly auto-paracrine role of BDNF for supporting growth and differentiation of mesencephalic dopaminergic neurons. However, a classic target-derived mechanism has been postulated for the critical period of dopamine neuron development (Lindsay et al. 1993), during which relatively high striatal BDNF levels could be detected.

A huge body of work has been devoted to elucidating the ability of the neurotrophins to promote survival, differentiation and regeneration of nigral dopaminergic neurons owing to the important role the degeneration of these neurons plays for the motor symptoms in Parkinson's disease. BDNF was the first member of the neuro-

trophin family found to exert neurotrophic effects on dopaminergic neurons of the substantia nigra in vitro (Hyman et al. 1991; Knüsel et al. 1991). In addition, BDNF has been shown to protect dopaminergic neurons of rat mesencephalon against various neurotoxic agents (Spina et al. 1992; Skaper et al. 1993; Altar et al. 1994). This suggests the possibility that BDNF may directly interfere with the neurodegenerative process that causes the destruction of dopaminergic neurons in the substantia nigra of Parkinson's disease patients.

In recent years neural transplantation has emerged as a potential therapeutic tool in severe Parkinson's disease, and more than 150 patients have been transplanted with dopamine-rich human fetal mesencephalic grafts (for review, Lindvall 1994). Although beneficial effects of these operations have been reported in several cases (Lindvall et al. 1990; Freed et al. 1992; Lindvall et al. 1992; Widner et al. 1992), the degree of clinical recovery has remained limited so far. The number of surviving dopaminergic neurons is believed to be one crucial factor for successful grafting in patients, as previously demonstrated in various animal models of Parkinson's disease (Brundin et al. 1988; Spector et al. 1993). Pre-, peri- or postoperative application of neurotrophic factors to fetal mesencephalic grafts may increase the number and function of surviving dopaminergic neurons and thereby help to improve clinical outcome.

There are only a few studies investigating the effects of neurotrophic agents on human mesencephalic tissue. Survival effects of BDNF have been detected in organotypic and free-floating roller tube cultures (Spenger et al. 1992, 1995) as well as in dissociated cultures of embryos at postconception week 6–10 (Zhou et al. 1994; Othberg et al. 1995). A corresponding increase in phenotypic markers, including dopamine content and tyrosine hydroxylase (TH) activity, has been reported (Zhou et al. 1994; Spenger et al. 1995). Effects of BDNF on a single-cell level has remained inconclusive. The present study aims to extend the knowledge about in vitro effects of BDNF on human dopaminergic neurons: first, by assessing survival effects in tissue originating from embryos at postconception week 6–8; second, by carrying out an extensive analysis of morphological differentiation to give a detailed, quantitative characterisation of the neuronal structure of human dopaminergic neurons in vitro; and third, by demonstrating specific actions of BDNF on morphological differentiation.

Materials and methods

Preparation of dissociated cultures from human fetal ventral mesencephalon

Human fetal tissue was obtained with approval of the Research Ethical Committee at the Medical Faculty of the University of Lund from routine suction abortions. Informed consent was given by women seeking abortion. The age of the human fetuses was assessed by ultrasound, and the material used was from five different fetuses at postconception week 6–8 (crown-rump length 18–25 mm). Ventral mesencephalon was dissected out in Dulbecco's minimal essential medium (DMEM; Gibco, USA). Pooled tissue was incubated in 0.1% trypsin (Worthington Biologicals Corporation) and 0.05% DNase (DN-25; Sigma), at 37°C for 20 min, rinsed four times in 0.05% DNase and mechanically dissociated using a 1-ml automatic pipette. The tissue was then centrifuged at 600 rpm for 5 min and the pellet was resuspended in DMEM. The cell number was assessed with a trypan blue dye exclusion method and 100000 cells/cm^2 were plated onto four-well chamber slides (Nunc, Naperville, Ill., USA). The wells were precoated with 10 μg/ml poly-L-lysine (Sigma) and 2.5 μg/ml Merosin (Chemicon, Temecula, Calif., USA). Cells were left for 24 h (i.e. 1 day in vitro, 1 DIV) with high-glucose DMEM (Gibco) plus 10% heat-inactivated fetal calf serum (FCS; Gibco), 1 ml/well at 5% CO_2, 37°C and 95% humidity. After 1 DIV the culture medium was changed to serum-free N2 medium (Bottenstein et al. 1979), consisting of DMEM/Ham's F12 (1:1) mixture (Gibco) supplemented with 10 mM $NaHCO_3$ (Gibco), 2 mM l-glutamine (Gibco), 110 μg/ml sodium pyruvate (Gibco), 100 μg/ml transferrin (Sigma), 20 ng/ml insulin (Sigma), 100 μM putrescine (Sigma), 30 nM sodium selentite (Sigma), 20 μM progesterone (Sigma) and no antibiotics. BDNF was added to the medium from day 1 to 7 at a concentration of 50 ng/ml. This concentration has been shown to produce optimal survival and differentiation effects for dopamine neurons in dissociated cultures of rat ventral mesencephalon (Hyman et al. 1994). Cultures were maintained in vitro for 7 days.

Immunocytochemistry for TH

Cultures were rinsed for 5 min in phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 15 min at room temperature and washed three times with PBS. They were then quenched with 3% H_2O_2 for 7 min, washed three times with PBS, and preincubated with 5% normal swine serum (NSS; Sigma) and 0.1% Triton X-100 in PBS for 1 h at room temperature. The slices were then incubated with the primary TH antibody (1:500; Pel Freeze), 2% NSS and 0.1% Triton X-100 in PBS overnight at 4°C. Following three washes with Triton X-100 in PBS, the slides were incubated for 1 h at room temperature with biotinylated swine-anti-rabbit antibody (1:200; Dakopatt, Glostrup, Denmark) with 0.1% Triton X-100 in PBS. The cultures were washed three times with PBS. Labelling was visualised by incubation with Vectastain ABC Elite kit in PBS for 30 min at room temperature, washing three times with PBS, and finally incubating with 0.05% 3,3'-diaminobenzidine and 0.03% H_2O_2 in PBS, washing six times with PBS and mounting with glycerol/gelatine (Sigma).

Cell counting and computer-assisted reconstruction

Survival of dopaminergic neurons was assessed by counting all TH-immunoreactive (TH-ir) cells in each well blindly with respect to culture conditions. For reconstruction neurons were selected by a uniform random sampling procedure. Reconstructions of 120 neurons in the BDNF-treated group and 80 neurons in the control group were performed in a blinded manner by means of a Eutectic three-dimensional neuron tracing system (Capowski 1989) as described previously (Studer et al. 1994). Briefly, the system consists of: an IBM-compatible AT-386 computer; a Eutectic vector display processor, model 3 (VDP3); a Nikon Optiphot-2 microscope, with a motorised stage and a Nikon ×1.25 drawing tube; a Eutectic joystick-box, with integrated motor controller; a large-screen Eutectic cathode ray tube; and an HP Laserjet IIIp printer. Reconstructions were made with bright-field illumination. The final magnification was ×400 (×40 objective; numerical aperture 0.65).

Morphological criteria

Criteria to be met by each blindly and uniformly randomly selected neuron for inclusion in the reconstruction analysis have been

described in detail previously (Studer et al. 1995). Briefly, only strongly TH-ir neurons were reconstructed (all questionable cases were excluded), morphological structure had to be fully intact and the neurons had not to overlap with other TH-ir cell bodies. To prevent any bias among treatment groups, all TH-ir cells fulfilling these criteria, were eligible for reconstruction, independent of staining density, size and complexity. The term "neurite", encompassing both axons and dendrites, was used, because there were no reliable morphological criteria to distinguish between axons and dendrites in these cultures.

Quantitative parameters

Two groups of morphological parameters were analysed: first, parameters related to the proximal portions of neurons, namely cell soma size expressed as the *soma profile area* and the number of *stemneurites*, emerging from an individual cell soma; second, parameters describing neuritic size and complexity, including *combined neuritic length* and *volume*, the mean *number of segments* and *branch points*, the *maximal branching order* corresponding to the highest topological path length (Uylings et al. 1986) plus 1, the *combined neuritic field area* and, finally, the *Sholl's concentric sphere analysis* (Sholl 1956), allowing determination of complexity changes at distances in 10-mm steps from the midpoint of the cell soma. Neuritic field area was determined on a per-neuron basis by fitting over each tree the smallest possible convex polygon crossing all the peripheral endings in the *xy*-axis. The area inside the polygon was calculated using the trapezoidal rule and was summed up for all trees of a neuron. Detailed descriptions of all the other parameters investigated have been given previously (Studer et al. 1994). Due to the low number of TH-ir neurons with spines or varicosities and due to the generally low neuritic complexity, no additional parameters of differentiation or basic architecture were included in the analysis.

Statistical analysis

A commercially available statistical software package (Systat 5.0; Systat, Evanston, Ill., USA) was used for statistical analysis. Data comparing survival of dopaminergic neurons effects were tested by one-way analysis of variance (ANOVA) and Scheffé's post hoc test. Proximal morphological parameters and parameters of length and complexity were analysed by the two-sided, non-parametric Mann-Whitney *U*-test. All results in the text and in the graphs are given as the mean±SEM.

Results

Qualitative description of TH-ir neurons in dissociated cultures of fetal human mesencephalon

Following immunocytochemistry, TH-ir neurons could be detected throughout the culture plate (Fig. 1). The percentage of TH-ir cells compared with the total cell number as determined in phase-contrast microscopy was between 2 and 5%. Sometimes, TH-ir neurons formed clusters of up to 20 cells each. Most TH-ir cells displayed an ovaloid spindle-shaped soma. Stemneurites preferentially emerged from basal and the apical parts of the soma, leading to a bipolar orientation of the neurons. Neurites were smooth, scarcely branching and devoid of spines and varicosities. Tortuosity, describing the meandering of neuritic segments from branchpoint to branchpoint, was generally low, and no obvious difference in neuronal structure between BDNF-treated and untreated neurons was visible. Only a small percentage of all TH-ir neurons were undifferentiated and displayed no processes at all.

Comparison of the number of surviving TH-ir cells between BDNF-treated and untreated neurons

We have previously demonstrated increased survival of human mesencephalic dopaminergic neurons in BDNF-treated dissociated cultures (Othberg et al. 1995) and in solid tissue cultures (Spenger et al. 1992; 1995). This effect was confirmed here for dissociated cells in two additional culture sets. The mean number of TH-ir neurons per well in the first set was 768±51 (n=4) and in the second set, 1344±362 (n=4) in control cultures, and in the first set was 1507±155 (n=4) and in the second set, 2210±214 (n=4) in BDNF-treated cultures (for each culture set P<0.05).

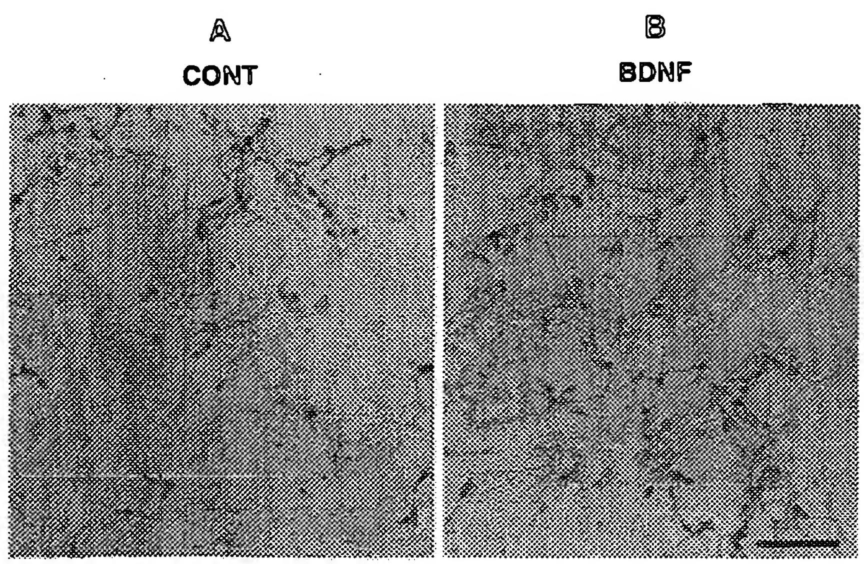


Fig. 1A, B Dissociated cultures of human fetal ventral mesencephalon 7 days in vitro, stained for tyrosine hydroxylase (TH). **A** Culture was maintained in vitro without BDNF supplementation. **B** Brain-derived neurotrophic factor (BDNF, 50 ng/ml) was added to the culture medium.The number of TH-immunoreactive neurons is slightly increased in BDNF-treated cultures as compared to control cultures. *Scale bar* 100 µm; objective ×10, bright-field illumination

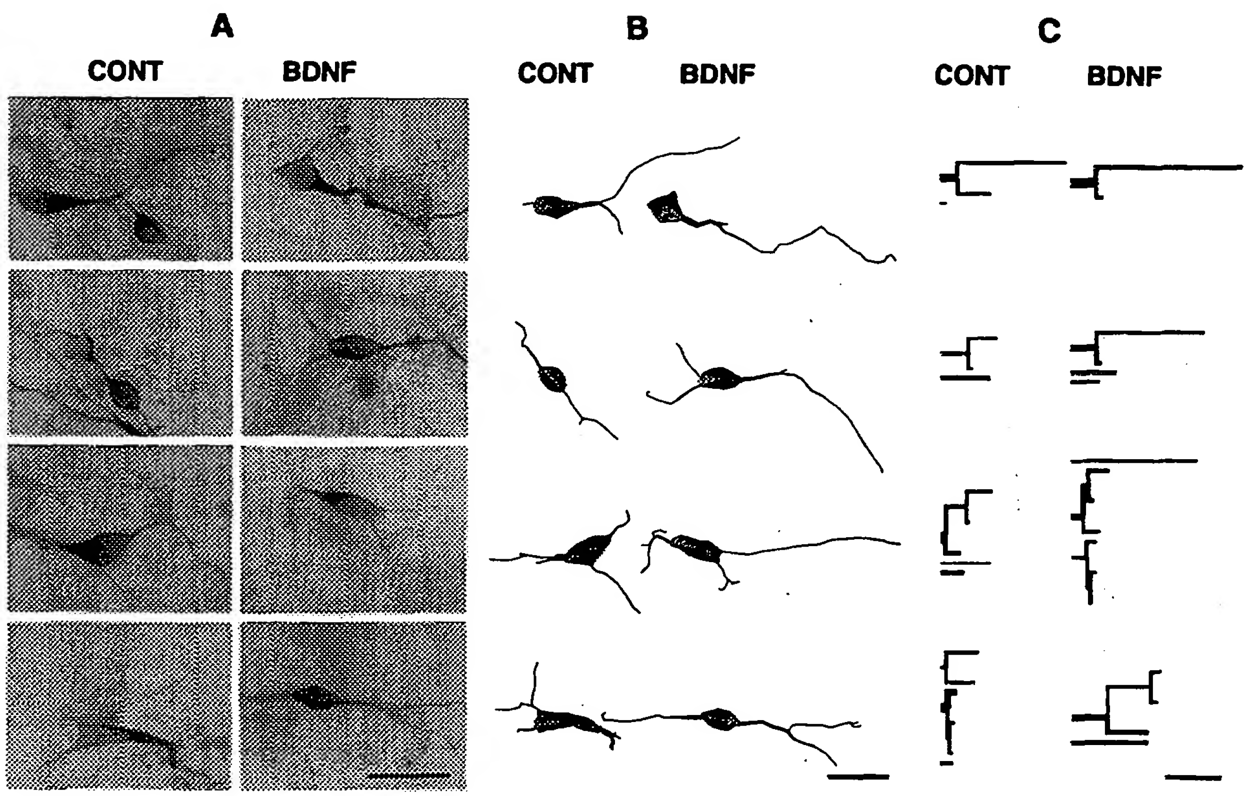
Quantitative comparison of the morphological structure of TH-ir cells between BDNF-treated and untreated cultures

A systematic morphometric analysis was carried out to show effects of BDNF on soma size and neurite outgrowth. The proximal sections of four representative neurons each in the BDNF group and the control group are displayed in Fig. 2A. Several TH-ir fibres in both groups in Fig. 2A do not belong to the neuron selected for reconstruction but are portions of neurites of other TH-ir neurons located nearby. Figure 2B illustrates the reconstruction of the complete neuronal structure of the cells presented in Fig. 2A. Figure 2C shows the corresponding dendrograms, which schematically illustrate the branching pattern of a neuron. Number of segments, branch points, and branch orders, segment length and distances from a tree origin to its endings in the periphery are represented. Neuritic trees are ranked for each neuron according to their combined neuritic length. In addition, the classic model of a dendrogram was modified to include mean neuritic thickness of each segment by drawing thickness of all the respective segments in the dendrogram proportionally. A relationship between neuritic thickness at the root segment and the total length and complexity of the corresponding neurites, a finding previously reported for motoneurons (Ulfhake and Cullheim 1981), can be confirmed in our cultures for TH-ir cells (Fig. 2C). A tendency towards longer and more complex neurites in BDNF-treated neurons can be detected in Fig. 2B and C.

Fig. 2 **A** Photomicrographs of the proximal zone of four representative TH-immunoreactive neurons of the control and the BDNF-treated groups. Location and orientation of the cells correspond to those of the plots and graphs in **B** and **C**. No clear differences can be detected between neurons of the two groups without further quantification. Objective ×40, bright-field illumination. **B** Computer-assisted reconstructions of the eight neurons in **A**. These show the complete neuritic arbors of the cells, revealing slightly longer neurites in BDNF-treated neurons than in the control group. **C** Dendrograms of the same eight neurons; *y*-axis is chosen arbitrarily. The *top* representation of each neuron is the tree with the maximal distance between tree origin and peripheral ending. Thickness of each tree is proportional to the mean thickness of the respective segment. *Scale bars* 25 μm

Effects of BDNF on parameters of the proximal zone of a neuron

Quantitative analysis of the reconstructed images of the cultured TH-ir neurons was carried out for 120 neurons of the BDNF group (80 neurons of culture set 1; 40 neurons of set 2) and 80 neurons of the control group (40 neurons of culture set 1; 40 neurons of set 2). The percentage of uniform, randomly chosen and reconstructed neurons in relation to the total TH-ir cell population was between 2.6 and 5.2%. A first class of parameters investigated was that related to the proximal zone of TH-ir neurons, namely soma profile area and mean number of stemneurites. Soma profile area was significantly increased by 20% and 14% in culture sets 1 and 2, respectively, and by 18% ($P<0.001$; Fig. 3A) in the pooled analysis. There was no significant difference between the values of culture set 1



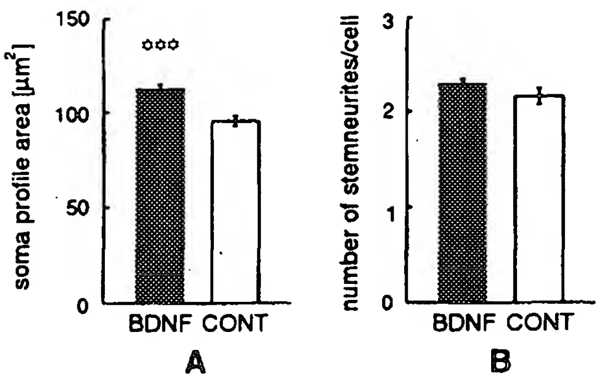


Fig. 3 A Soma profile area. Values are mean±SEM per group. ***Significance from value of control group, $P<0.001$. **B** Number of stemneurites. Values are mean±SEM per group. No significant differences could be detected. BDNF-treated neurons, $n=120$; control neurons, $n=80$

and that of set 2. The mean number of stemneurites was not affected by BDNF treatment either in culture set 1 or 2, nor in the pooled analysis as given in Fig. 3B.

Effects of BDNF on parameters of neuritic size and complexity

Additional analysis of the reconstructed neurons was performed to investigate the effects of BDNF on parameters relating to neuritic size and complexity. In BDNF-treated neurons the neuritic volume was increased by 125% and the neuritic length by 60% as compared to controls (Fig. 4B, C; pooled analysis). As the increase in neuritic volume is double of that of the neuritic length, an increased mean neuritic thickness is expected in BDNF-treated cultures. In addition, a significant increase in the mean number of neuritic segments could be detected (Fig. 4A; pooled analysis). The increase in maximal branch order as shown in Fig. 4D (pooled analysis) illustrates that BDNF-induced branching also takes place at the most complex tree of TH-ir cells. Furthermore, BDNF elicited a significant increase in mean neuritic field area by 128% (Fig. 4E; pooled analysis). In contrast, no significant elevation in the mean segment length was observed: BDNF-treated neurons 30±2 µm, $n=120$; controls 28±2 µm, $n=80$ (P not significant). All values for the parameters illustrated in Fig. 4A-E were significantly increased under BDNF treatment in culture sets 1 and 2, except the mean number of segments, which did not reach statistical significance in culture set 2 (16% increase in BDNF-treated neurons; P not significant).

The generally higher degree of neuronal complexity in BDNF-treated neurons, as demonstrated in Fig. 4, was further characterised by the Sholl's concentric sphere analysis (Fig. 5; pooled analysis). At radial distances between

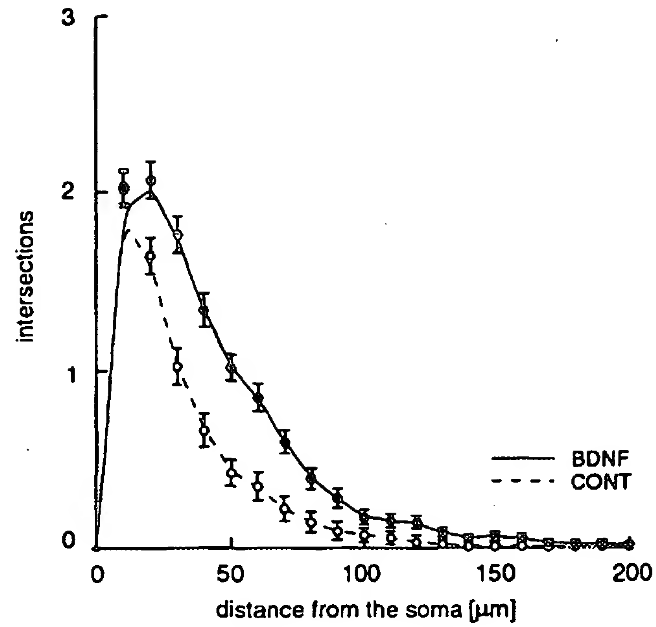
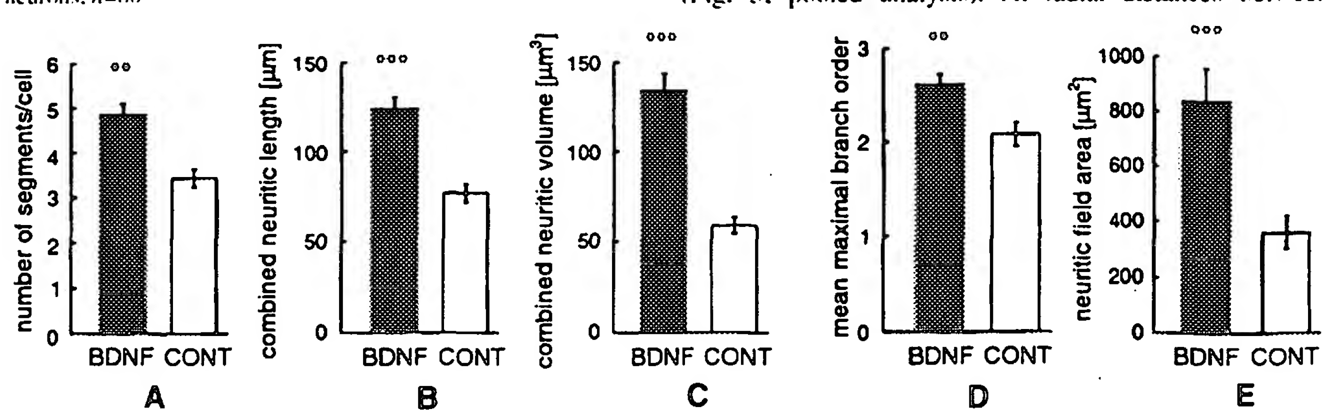


Fig. 5 Sholl concentric sphere analysis. Values are mean±SEM of the number of neuritic intersections per cell for control and BDNF groups in relation to the radial distance from the nucleolus, binned for every 10 µm. Data are fitted by distance-weighted least-square method with a tension factor of 0.001. BDNF-treated neurons, $n=120$; control neurons, $n=80$ for all distances from the soma

Fig. 4 A Mean number of neuritic segments per cell. **B** Combined neuritic length. **C** Combined neuritic volume. **D** Mean maximal centrifugal branch order. **E** Mean neuritic field area per cell. **A–E** Values are mean±SEM. Significance from value of control group: **$P<0.01$; ***$P<0.001$. BDNF-treated neurons, $n=120$; control neurons, $n=80$



20 μm and 130 μm from the soma midpoint significant increases in neuritic complexity were found suggesting that BDNF either is able to elicit new segments in this region or helps to sustain a generally higher tree complexity under culture conditions. Very close to the soma (defined as a radial distance of less than 20 μm from the soma midpoint) no difference in the mean number of intersections was seen. This is in agreement with an unchanged number of stemneurites in BDNF-treated neurons (see Fig. 3B)

Discussion

A huge body of in vitro work has been devoted to describe the influence of the various members of the neurotrophin family on biochemical and morphological parameters of fetal rat dopaminergic neurons. BDNF has been found to significantly increase high-affinity dopamine uptake (Beck et al. 1993; Hyman et al. 1994) and dopamine transporter [^{3}H]mazindol (Studer et al. 1995) in dissociated cultures. In addition, morphological parameters such as soma profile area and the mean number of stemneurites per neuron were consistently increased in rat dopamine neurons under BDNF treatment (Beck et al. 1993; Studer et al. 1995). Furthermore, measures of tree size and complexity were only slightly influenced by BDNF (Studer et al. 1995). No gross anatomical abnormalities have been observed in mice carrying a deletion of the BDNF gene (Ernfors et al. 1994; Jones et al. 1994). With respect to a potential role of BDNF in new therapeutic approaches in Parkinson's disease, it is of the utmost importance to confirm the results obtained with BDNF in rodent tissues (for review, Lindsay et al. 1993) in experiments using human tissue. BDNF could be used in a clinical setting either to support survival and phenotypic differentiation of transplanted human fetal dopaminergic neurons or by directly interfering with the degenerative process leading to the destruction of dopaminergic neurons in the substantia nigra pars compacta. In human tissue, BDNF has been shown to influence dopamine and serotonin content as well as TH and glutamic acid decarboxylase (GAD) activity (Zhou et al. 1994; Spenger et al. 1995). These increases are paralleled by enhanced survival and fibre density of dopaminergic neurons, a finding already described earlier (Spenger et al. 1992; Othberg et al. 1995). Effects of BDNF at a single-cell level have been demonstrated in the present study by a comparative, detailed morphometric characterisation of neuronal structure. BDNF has been found to not only increase survival of dopaminergic neurons in dissociated cultures of human fetal ventral mesencephalon but also specifically act on various parameters of size, complexity and branching pattern of individual dopaminergic neurons.

General morphological features of human dopaminergic neurons in vitro

Dopaminergic neurons of the human fetal ventral mesencephalon exhibit different levels of maturity in dissociated cultures. Such differences in maturity may be due to region-specific variations of the developmental stage of neurons (Olson and Seiger 1972; Pickel et al. 1980; Specht et al. 1981). As shown previously by Pickel et al. (1980) and Freeman et al. (1991), precursor cells of dopaminergic neurons start to express TH at postconception week 6.5 in the anlage of the substantia nigra, and neurite formation begins at 8 weeks. In the present study, dopaminergic neurons were obtained between postconception week 6 and 8 and were maintained in vitro for 1 week. In dissociated cultures of human ventral mesencephalon, neuritic length and complexity was low compared with what has been observed in dissociated cultures of fetal rat mesencephalon (Beck et al. 1993; Studer et al. 1995). This may be due to the fact that developmental speed is much slower in the human than in the rat CNS, where, theoretically, neonatal age is reached after only 1 week in vitro for embryonic day 14 neurons, whereas human dopaminergic neurons at postconception week 6–8 are still at embryonic level after 1 week in vitro. The percentage of 2–5% dopaminergic neurons of the total number of mesencephalic cells in the culture system was similar to the percentage obtained with rat tissue (Hyman et al. 1991, Beck et al. 1993). No staining-density measurements have been performed in the present study. However, there was no obvious difference in the TH immunoreactivity between BDNF-treated and control neurons (see Fig. 2A). Terminal arborizations of neurons were also checked using phase-contrast microscopy in order to avoid possible errors due to differences in staining intensities.

Morphological parameters related to the proximal zone of a neuron

The soma profile area in human fetal neurons in dissociated cultures, as presented in this study, was significantly smaller than that in rat fetal neurons after the same time in vitro (Beck et al. 1993; Studer et al. 1995). However, similar relative increases in BDNF-treated and untreated neurons were seen in both rat and human cultures. This shows an equivalent potency of BDNF for this parameter in both species. Interestingly, the emergence of new stemneurites from human dopaminergic neurons was not affected by BDNF treatment. In contrast, in rat tissue increases up to fourfold have been reported (Beck et al. 1993). Differences in the developmental clock or in culture conditions may account for these observations.

Morphological parameters related to neuritic length and complexity

Effects of BDNF on neuritic branching have been demonstrated for cholinergic neurons of the basal forebrain (Alderson et al. 1990; Knüsel et al. 1991), GABAergic neurons of the striatum (Ventimiglia et al. 1995) and

cerebellum (Segal et al. 1992), motoneurons (Henderson et al. 1993), retinal ganglion cells (Cohen et al. 1994) and for dopaminergic neurons of the mesencephalon (Beck et al. 1993; Studer et al. 1995). To date, there have been no quantitative studies detailing effects of BDNF on neuritic growth, complexity and the branching patterns of dopaminergic neurons of human fetal mesencephalon. Increases in the number of branch points and neuritic segments as well as in combined neuritic length and volume, as demonstrated in the present study, suggest that BDNF either leads to an increased branching rate or allows maintenance of a generally higher tree complexity in vitro. Combined neuritic volume was, together with the neuritic field area, the morphometric factor most clearly affected by BDNF treatment. This may be due to the fact that not only the mean number of segments and branch points but also the segment length and thickness is reflected by this parameter. Relative increases in the complexity of both human and rat dopaminergic neurons by BDNF treatment were very similar (Studer et al. 1995). As evidenced by the Sholl concentric sphere analysis, changes in complexity could be detected at all distances relative to the soma midpoint, suggesting that BDNF led to an equally enhanced growth mode and branching probability for all neuritic parts independent of their localisation within the arbor. Interestingly, the Sholl's concentric sphere analysis in rat cultures showed a more bimodal BDNF-induced growth pattern for the same type of neurons, with increases in complexity localised either close to the soma (<60 μm) or at the periphery (>140 μm; Studer et al. 1995). However, comparisons of such data have to be interpreted with caution, because non-physiological growth patterns due to culture conditions which affect human and rat tissue differently cannot be ruled out. In addition, it was not possible in the present study to distinguish between axons and dendrites, which can be differently influenced by neurotrophin treatment (Studer et al. 1994). Therefore it cannot be excluded that different relative proportions of axonal to dendritic arbors of human and rat neurons lead to discrepancies in the neuritic growth mode analysed.

In conclusion, this study demonstrates that BDNF influences not only survival but also size and complexity of dopaminergic neurons of human fetal mesencephalon in vitro. Further work will have to elucidate the time and dose responsiveness as well as the receptor mechanisms involved in these morphological changes. It will be interesting to explore whether other factors with trophic effects on rat neurons, such as NT-4/5 (Studer et al. 1995), or members of the transforming growth factor-β superfamily, such as glial cell line-derived neurotrophic factor (GDNF; Lin et al. 1993; Cheng et al. 1995; Johansson et al. 1995), may have even more robust and wide-ranging effects on the morphological differentiation of human dopaminergic neurons. The subtle but consistent BDNF-induced changes of neuronal structure, barely detectable without a systematic quantitative analysis, underscore the necessity to perform morphological studies about the effects of neurotrophic factors and neuronal cytoarchitecture in null mutant mice with great care. The BDNF effects on survival and differentiation of single human fetal dopaminergic neurons at the optimal age for neurotransplantation support a role for BDNF in new therapeutic approaches in Parkinson's disease.

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